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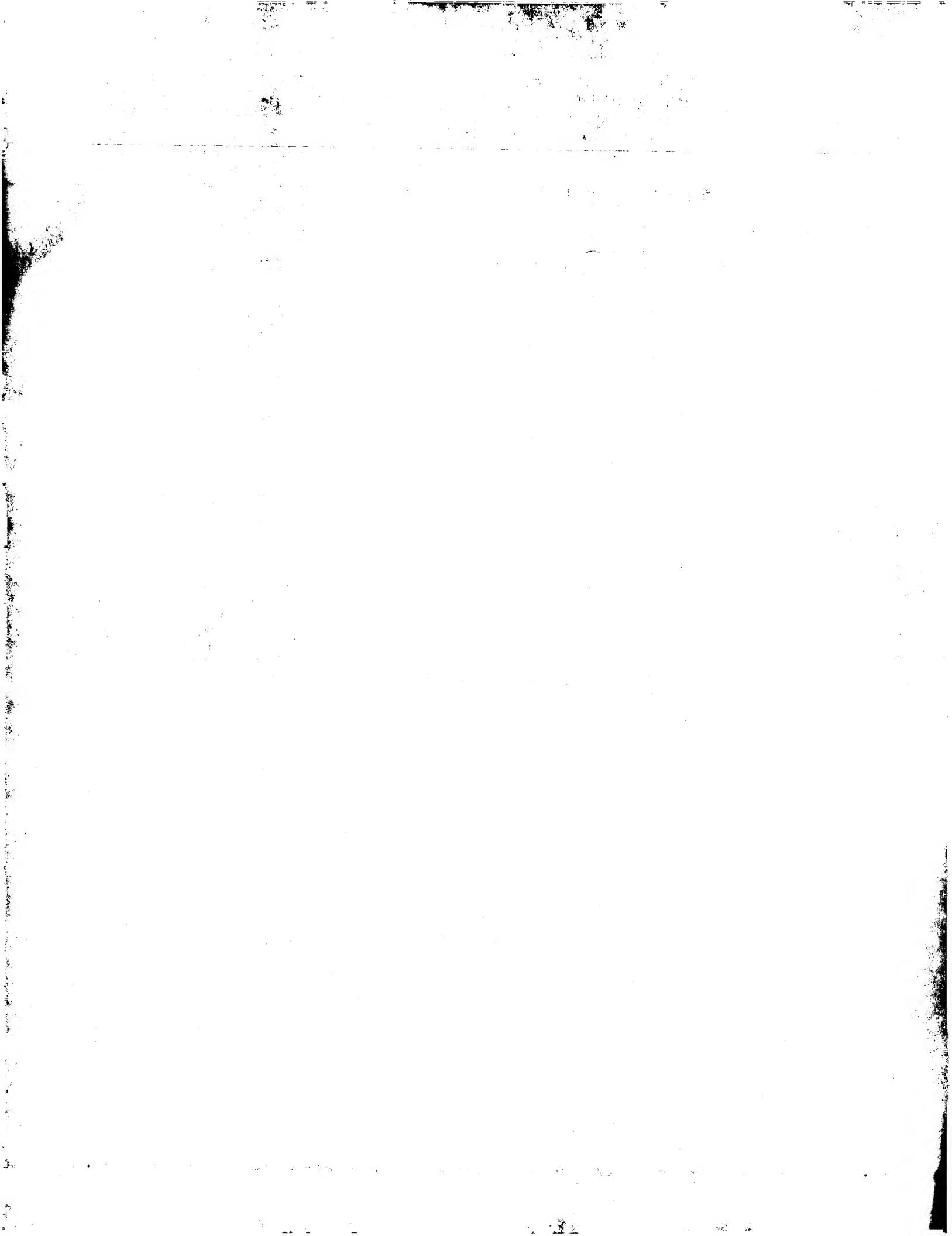
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(54) Title: TREATMENT OF MICROBIAL INFECTIONS WITH BACTERIAL PROTEINS AND PEPTIDES

(57) Abstract: The present invention relates to protein and peptide chemistry. In particular, the present invention relates to the discovery and isolation of novel peptides whose sequences derive from microorganism by-products including biosurfactants. The invention is also directed to the use of these novel peptides in the inhibition of infections caused by pathogens.

TREATMENT OF MICROBIAL INFECTIONS WITH BACTERIAL PROTEINS AND PEPTIDES

FIELD OF THE INVENTION

The present invention relates to protein and peptide chemistry. In particular, the present invention relates to the discovery and isolation of novel peptides whose sequences derive from microorganism by-products including *Lactobacillus* biosurfactants. The invention is also directed to the use of these novel peptides in the inhibition of infections caused by pathogens.

BACKGROUND OF THE INVENTION

Microorganisms still represent one of the top three causes of death amongst humans and animals. The offending organisms can be bacteria, fungi, protozoa, viruses and other forms, collectively termed as pathogens (or organisms which behave like pathogens under certain situations, such as when the host is immunocompromised). Pathogens use many different factors to cause disease, including adhesins to colonize tissues and surfaces, toxins, slime and other capsular substances, antibiotic-resistance genes, immune modifiers and substances which help escape immune responses, etc.

The primary exogenous mechanism to eradicate offending pathogens is antimicrobial agents, such as antibiotics. However, these are often ineffective due to resistance of pathogenic organisms, inability to eradicate biofilms and poor penetration at the tissue or biomaterial interface.

There are a number of organisms which can infect the host. Of these, *S. aureus* (particularly

methicillin-resistant - MRSA), *S. epidermidis*
(particularly methicillin-resistant - MRSE) , *Pseudomonas
aeruginosa, Enterococcus faecalis* (including vancomycin-
resistant - VRE) and *Bacteroides sp.* can be particularly

5   detrimental in wounds and surgical implants. These and
other aerobic and anaerobic pathogens cause severe
morbidity and death in large patient populations.

Implanted medical devices, such as heart valves
and artificial veins and joints, are especially

10   vulnerable to microbial biofilm formation and disease
(Gristina, (1987) <u>Science</u>, 237:1588-1595). These
surfaces are not well protected by host defenses and such
surfaces provide a focal point for infecting pathogens.
Closed implants are more frequently associated with life-

15   threatening morbidity. *Staphylococcus epidermidis* and *S.
aureus* being the major pathogens associated with life-
threatening infections (Christensen et al. (1989)
"Microbial and foreign body factor in the pathogen of
medical device infections. In infections associated with

20   in dwelling medical devices", Bisno AL, Waldvogel FA
(eds), ASM, Washington, pp.2759). Thus, various
microorganisms share a common ability to form biofilms
and colonize human tissues in the presence and absence of
foreign bodies.

25   To combat the significant infectious
complication rates associated with the use of surgical
implants, several strategies have been employed. Thus
far, these have been mainly limited to:improved surgical
asepsis techniques, improved regimens of administration

30   of peri-operative systemic antibiotics, local antibiotic
irrigation procedures, modification of surface

characteristics of surgical implants and impregnation of
surgical implants with antibiotics.

Thus, progress has been made, but that based on
scientific innovation, has been relatively slow, and most
citations in the literature still utilize traditional
methods of management, such as use of antibiotics,
antiseptics and surgical debridement. Newer approaches to
prevent bacteria ascending towards clinical use include
vacuum sealed and transparent film dressings, irrigation
with antimicrobial agents, use of the port and cap, use
of new agents such as deuteroporphyrin against
Staphylococcus aureus, gamma interferon, silver
sulfadiazone water soluble gel, geomagnetic therapy, and
natural remedies such as milliacynic oil and lysozyme.
However, few innovations have made a major impact on
infection and fatality rates. Indeed, most new approaches
involve delivery of an antimicrobial chemical, for
example in liposomes or in collagen dressings.

SUMMARY OF THE INVENTION

One embodiment of the present invention is
directed to microorganism biosurfactant peptides. In a
preferred embodiment the microorganism is *Lactobacillus*.
In another preferred embodiment the microorganism is
Staphylococcus epidermidis. The present application also
contemplates biosurfactant peptides isolated from
Streptococcus sp., *Bifidobacterium sp.* and lactic acid
bacteria. In accordance with the present invention,
novel peptides derived from or corresponding to by-
products produced by *Lactobacilli* have been isolated and
synthesized. These peptides possess anti-microbial

properties including the ability to selectively bind to collagen and inhibit infections around wounds and at the site of implants and biofilms which biofilms, for example, are associated with infections in mammals, *in vivo*. The peptides of the present invention are derived from human microorganism isolates and therefore are non-immunogenic, non-pathogenic, non-carcinogenic and non-invasive when administered to humans.

The present invention also provides methods and compositions for treating infections such as are commonly caused by *S. aureus* (particularly methicillin-resistant - MRSA), *S. epidermidis* (particularly methicillin-resistant - MRSE) , *Pseudomonas aeruginosa, Enterococcus faecalis* (including vancomycin-resistant - VRE) and *Bacteroides sp.*, for example.

The present invention also provides methods and compositions for coating devices, including stents, tubes, prosthetics, bandages, intravenous lines, catheters, diaphragms, tampons and the like. The present invention provides compositions containing at least one biosurfactant peptide which inhibits, prevents, treats or reduces the risk of infections in a mammal, and preferably humans.

The present invention also provides a method to identify proteins, peptides, glycoproteins and amino acid sequences from any given microorganism which block the lock and key adherence of pathogens, such as but not limited to *Salmonella, Shigella, E. coli, Pseudomonas, Listeria, Yersinia, Enterococcus, Streptococcus* and other pathogens including viruses and protozoa.

The biosurfactant peptides of the present invention are at least 5 amino acids in length and possess collagen-receptor binding activity. Collagen is a major extracellular matrix (ECM) component of tissues that has also been shown to be an important site of attachment for invading pathogenic bacteria, like *Staphylococcus aureus*.

A preferred biosurfactant peptide of the present invention is a peptide having the sequence Xaa_1-Gln-Gly-Xaa_2-Val-Ser-Xaa_3-Xaa_4-Gly-Ser-Cys-Thr-Cys-Ala-Gln-Lys (SEQ ID NO:1), wherein Xaa_1 and Xaa_2 can be any amino acid.

Preferably, Xaa_1 and Xaa_2 are Leu. Xaa_3 and Xaa_4 can either be Phe or Ala. Another preferred biosurfactant peptide of the present invention is a peptide having the sequence Glu-Val-Tyr-Xaa_1-Phe-Gln-Glu-Xaa_2-Xaa_3-Arg (SEQ ID NO:2), wherein Xaa_1 can be any amino acid and Xaa_2 and Xaa_3 can either be Gln or Pro. Another preferred biosurfactant peptide of the present invention is a peptide having the sequence Trp-Asn-Tyr-Val-Ser (SEQ ID NO:3). Still another preferred biosurfactant peptide of the present invention is a peptide having the sequence Arg-Ala-Phe-Ala-Xaa_1-Xaa_2-Ser-Asn-Trp-Pro-Val-Lys (SEQ ID NO:4), wherein Xaa_1 and Xaa_2 can be any amino acid. Yet another preferred biosurfactant peptide of the present invention is a peptide having the sequence Gly-Xaa_1-Gln-Xaa_2-Xaa_3-Asp-Xaa_4-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Arg (SEQ ID NO: 5), wherein Xaa_1, Xaa_2, Xaa_3 and Xaa_4 can be any amino acid and Xaa_5, Xaa_6, Xaa_7, and Xaa_8 can be either Ser or His. Homologs and analogs of these peptides are also contemplated by the present invention.

Further, according to the present invention a biosurfactant peptide can be at least 5 amino acids and composed of up to a full length biosurfactant protein, as long as the peptide binds to a collagen receptor and

5 possesses anti-pathogenic activity.

Another aspect of the present invention provides methods of interfering with, blocking or otherwise preventing the interaction or binding of microbial pathogens to collagen receptors on cells, for

10 example, by employing the biosurfactant peptides contemplated by the present invention. As such the present peptides are understood to act as microbial antagonists.

Another aspect of the present invention

15 provides methods of interfering with blocking or otherwise preventing the interaction or binding of microbial pathogens to extracellular matrix substances on cells or on the outer surface conditioning film of a biomaterial, for example.

20 The present invention also provides compositions for the treatment of infections such as occur at wound sites, implant sites and the like in animals, including humans and includes methods of treating such disorders. The compositions include at

25 least one of the biosurfactant peptides, preferably at least the biosurfactant peptide according to the present invention, is admixed with a pharmaceutically acceptable carrier.

Nucleic acid molecules coding for any of the

30 above biosurfactant peptides of the present invention, expression vectors which include any of such nucleic acid molecules, as well as related host cells containing such

nucleotide sequences or vectors, are also contemplated by the present invention.

These and other embodiments of the invention will be readily apparent to those of ordinary skill in view of the disclosure herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1A is a SDS-PAGE gel of the flow through (FT), wash (W) and elution fraction (EF) of the Cn-I and Cn-III affinity columns.

FIGURE 1B is a bar graph demonstrating that the collagen binding activity of *L. fermentum* RC-14 biosurfactant inhibits *S. aureus* infection and bacterial adhesion to silicone implants *in vivo*, P=0.0039 (infection rate); P<<0.0001 (Implant CFUs).

FIGURE 2 is a SELDI protein spectrum of the RC-14 biosurfactant that bound to a Cn-III/PS-1 array. Arrows highlight distinct collagen binding peaks. Dual charged peaks $(m + 2H^+)$ and their potential single charged peaks $(m + H^+)$ are bracketed.

FIGURE 3A is a SELDI protein spectrum of the tryptic profiles of collagen binding fractions of *L. fermentum* RC-14.

FIGURE 3B is a SELDI protein spectrum of the tryptic profiles of collagen binding fractions of *L. rhamnosus* GR-1.

FIGURE 3C is a MS/MS spectra of a Lactobacillus biosurfactant peptide derived from *L. fermentum* RC-14, 1191,569 Da.

FIGURE 3D is a MS/MS spectra of a *Lactobacillus* biosurfactant peptide derived from *L. fermentum* RC-14, 1308.635 Da.

FIGURE 3E is a MS/MS spectra of a *Lactobacillus* biosurfactant peptide derived from *L. fermentum* RC-14, 1465.731 Da.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to biosurfactant peptides. In a preferred embodiment the peptides are isolated from *Lactobacillus* biosurfactants. The present invention also contemplates peptides isolated from *Staphylococcus sp.*, *Streptococcus sp.*, *Bifidobacterium sp.* and lactic acid bacteria. As defined by the present invention, a biosurfactant is a compound released by microorganisms with a tendency to accumulate at interfaces, most notably the liquid-air interface. By "biosurfactant peptides" is meant any molecule that inhibits, prevents, treats or reduces the risk of infections around wounds and at the site of biologically compatible implants by, e.g., interfering with, blocking the receptors onto which pathogens would otherwise bind including cells and biologically compatible devices. As used herein a "biologically compatible device" may be composed of polymers such as fluorinated ethylene propylene, sulfonated polystyrene, polystyrene, polyethyleneterephthalate silicone, polyurethane, polyvinylchloride silicone rubber, or glass, for example. The biodevice may be a catheter such as a urinary or peritoneal catheter, a diaphragm, a stent, an IUD or a diaper, an intravenous line, a peritoneal dialysis tube, a bandage, an endotracheal tube, or an intravaginal,

intrauterine, or intraurethral or intraurethral device, for example.

As used herein, "peptide" refers to a linear series of amino acid residues linked to one another by peptide bonds between the alpha-amino and carboxy groups of adjacent amino acid residues. The term "synthetic peptide" is intended to refer to a chemically derived chain of amino acid residues linked together by peptide bonds. The term "synthetic peptide" is also intended to refer to recombinantly produced peptides in accordance with the present invention.

The sequences of the peptides of the present invention are isolated from a biosurfactant, e.g., *Lactobacillus*. A preferred *Lactobacillus* is *L. fermentum* RC-14. However, peptide analogs derived from other Lactobacilli, including but not limited to *L. rhamnosus* GR-1 and other microorganisms are also encompassed by the invention. In the practice of the compositions and methods of the present invention, the biosurfactant peptides may be isolated from any microorganisms, in particular *Lactobacillus* species, especially *L. fermentum*, *L. acidophilus*, *L. reuteri*, but also potentially also *L. rhamnosus*, *L. casei*, *L. crispatus*, *L. plantarum*, *L. paracasei*, *L. jensenii*, *L. gasseri*, *L. cellobiosis*, *L. brevis*, *L. delbrueckii*, *L. helveticus*, *L. salivarius*, *L. collinoides*, *L. buchneri*, *L. rogosae*, and *L. bifidum*. These proteins can also be isolated from *Staphylococcus sp.*, *Bifidobacterium sp.* and other organisms.

A biosurfactant peptide functions in at least two ways. While not wishing to be bound to a particular mechanism of action, the peptides of the present

invention can bind to or sequester collagen receptors
with sufficient affinity and specificity to substantially
interfere with, block or otherwise prevent binding of
pathogens, thereby inhibiting or preventing infections.

5   Alternatively, a biosurfactant peptide can act as a
signaling molecule which can turn off a pathogen's
virulence capability, thereby also inhibiting or
preventing infections. Additionally, analogs, and
fragments of the novel peptides provided herein are

10   included within the scope of the term "biosurfactant
peptide".

By "analogs" is meant peptides which differ by
one or more amino acid alterations, which alterations,
e.g., substitutions, additions or deletions of amino acid

15   residues, do not abolish the anti-pathogenic properties
of the relevant peptides.

Thus, an analog may comprise a peptide having a
substantially identical amino acid sequence to a peptide
provided herein and in which one or more amino acid

20   residues have been conservatively or non-conservatively
substituted. Examples of conservative substitutions
include the substitution of a non-polar (hydrophobic)
residue such as isoleucine, valine, leucine or methionine
for another. Likewise, the present invention

25   contemplates the substitution of one polar (hydrophilic)
residue such as between arginine and lysine, between
glutamine and asparagine, and between glycine and serine.
Additionally, the substitution of a basic residue such as
lysine, arginine or histidine for another or the

30   substitution of one acidic residue such as aspartic acid
or glutamic acid for another is also contemplated.
Examples of non-conservative substitutions include the

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substitution of a non-polar (hydrophobic) residue such as
isoleucine, valine, leucine, alanine, methionine for a
polar (hydrophilic) residues such as cysteine, glutamine,
glutamic acid, lysine and/or a polar residue for a non-

5   polar residue.

        The phrase "conservative substitution" also
includes the use of chemically derivatized residues in
place of a non-derivatized residues as long as the
peptide retains the requisite anti-microbial activity.

10   Analogs also include the presence of additional
amino acids or the deletion of one or more amino acids
which do not affect biological activity. In addition,
for purposes of the present invention, peptides
containing D-amino acids in place of L-amino acids are

15   also included in the term "conservative substitution."

        A preferred biosurfactant peptide of the
present invention is a peptide having the sequence Xaa$_1$-
Gln-Gly-Xaa$_2$-Val-Ser-Xaa$_3$-Xaa$_4$-Gly-Ser-Cys-Thr-Cys-Ala-
Gln-Lys (SEQ ID NO:1), wherein Xaa$_1$ and Xaa$_2$ can be any

20   amino acid. Preferably, Xaa$_1$ and Xaa$_2$ are Leu. Xaa$_3$ and
Xaa$_4$ can either be Phe or Ala. Another preferred
biosurfactant peptide of the present invention is a
peptide having the sequence Glu-Val-Tyr-Xaa$_1$-Phe-Gln-Glu-
Xaa$_2$-Xaa$_3$-Arg (SEQ ID NO:2), wherein Xaa$_1$ can be any amino

25   acid and Xaa$_2$ and Xaa$_3$ can either be Gln or Pro. Another
preferred biosurfactant peptide of the present invention
is a peptide having the sequence Trp-Asn-Tyr-Val-Ser (SEQ
ID NO:3). Still another preferred biosurfactant peptide
of the present invention is a peptide having the sequence

30   Arg-Ala-Phe-Ala-Xaa$_1$-Xaa$_2$-Ser-Asn-Trp-Pro-Val-Lys (SEQ ID
NO:4), wherein Xaa$_1$ and Xaa$_2$ can be any amino acid. Yet
another preferred biosurfactant peptide of the present

invention is a peptide having the sequence Gly-Xaa$_1$-Gln-
Xaa$_2$-Xaa$_3$-Asp-Xaa$_4$-Xaa$_5$-Xaa$_6$-Xaa$_7$-Xaa$_8$-Arg (SEQ ID NO: 5),
wherein Xaa$_1$, Xaa$_2$, Xaa$_3$ and Xaa$_4$ can be any amino acid and
Xaa$_5$, Xaa$_6$, Xaa$_7$, and Xaa$_8$ can be either Ser or His. Still
5   yet another preferred biosurfactant peptide of the
present invention is a peptide having the sequence Gly-
Xaa$_1$-Gln-Asn-Asp-Xaa$_2$-Xaa$_3$-Xaa$_4$-Xaa$_5$-Xaa$_6$-Arg (SEQ ID
NO:6), wherein Xaa$_1$ and Xaa$_2$ can be any amino acid and
Xaa$_3$ Xaa$_4$, Xaa$_5$ and Xaa$_6$ can be either Ser or His. The
10   phrase "any amino acid" is understood, in accordance with
the present application to include:

                     A = Ala = Alanine
                     R = Arg = Arginine
                     N = Asn = Asparagine
15   D = Asp = Aspartic acid
                     B = Asx = Asparagine or aspartic acid
                     C = Cys = Cysteine
                     Q = Gln = Glutamine
                     E = Glu = Glutamic acid
20   Z = Glx = Glutamine or Glutamic acid
                     G = Gly = Glycine
                     H = His = Histidine
                     I = Ile = Isoleucine
                     L = Leu = Leucine
25   K = Lys = Lysine
                     F = Phe = Phenylalanine
                     P = Pro = Proline
                     S = Ser = Serine
                     T = Thr = Threonine
30   W = Trp = Tryptophan
                     Y = Tyr = Tyrosine
                     V = Val = Valine

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Further, according to the present invention a biosurfactant peptide can be longer than 5 amino acids and composed of up to a full length biosurfactant protein.

5   As used herein, the term "substantially corresponds" is meant the degree of amino acid homology of at least about 60% homology, preferably at least about 70%, and more preferably at least about 75%, which degree is the similarity index calculated using the Lipman-

10   Pearson Protein Alignment program with the following choice of parameters: Ktuple=2, Gap penalty=4, and Gap Length Penalty=12.

The term "fragment" refers to any subject peptide having an amino acid sequence shorter than that

15   of any peptide depicted in SEQ ID NOS: 1-6 and which fragment retains the anti-pathogenic activity of the subject peptides.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of

20   synthetic organic chemistry, protein chemistry, molecular biology, microbiology, and recombinant DNA technology, which are well within the skill of the art. These techniques are applied in connection with peptide identification, synthesis, recombinant production of

25   peptides and peptide mutagenesis, for example. Such techniques are explained fully in the literature. See e.g., Scopes, R.K., Protein Purification Principles and Practices, 2d ed. (Springer-Verlag. 1987), Methods in Enzymology (M. Deutscher, ed., Academic Press, Inc.

30   1990), Sambrook, et al., Molecular Cloning: A laboratory Manual, 2d ed., (Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1989), Handbook of Experimental Immunology,

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Vols. I-IV (D.M. Weir and C.C. Blackwell, eds., 1986, Blackwell Scientific Publications), House, Modern Synthetic Reactions, 2d ed., (Benjamin/Cummings, Menlo Park, Cal., 1972).

5   The peptides of the present invention are screened within the biosurfactant, e.g. RC-14, using Surface Enhanced Laser Desorption/Ionization time of flight mass spectrometry (SELDI-TOF-MS). The peptides of the present invention, analogs and fragments thereof may

10   be synthesized by a number of known techniques. For example, the peptides may be prepared using the solid-phase synthetic technique initially described by Merrifield, in J. Am. Chem. Soc. 85:2149-2154 (1963). Other peptide synthesis techniques may be found in M.

15   Bodanszky, et al. Peptide Synthesis, John Wiley & Sons, 2d Ed., (1976) and other references readily available to those skilled in the art. A summary of polypeptide synthesis techniques can be found in J. Stuart and J.D. Young, Solid Phase Peptide Synthesis, Pierce Chemical

20   Company, Rockford, Ill., (1984). Peptides may also be synthesized by solution methods as described in The Proteins, Vol. II. 3d Ed., Neurath, H. et al., Eds., p. 105-237, Academic Press, New York, N.Y. (1976). Appropriate protective groups for use in different

25   peptide syntheses are described in the above-mentioned texts as well as in J.F.W. McOmie, Protective Groups in Organic Chemistry, Plenum Press, New York, N.Y. (1973). Additionally, the peptides of the present invention may also be prepared by recombinant DNA

30   techniques (see e.g. Current Protocols in Molecular Cloning Ausubel et al., 1995, John Wiley & Sons, New York); Sambrook et al., 1989, Molecular Cloning: A

Laboratory Manual, Second Edition, Cold Spring Harbor
Laboratory Press, New York; Coligan et al. *Current
Protocols in Immunology*, John Wiley & Sons Inc., New
York, New York (1994)). The skilled artisan understands
that any of a wide variety of expression systems can be
used to provide the recombinant peptides of the present
invention. The precise host cell used is not critical to
the invention. The biosurfactant peptides can be
produced in a prokaryotic host (e.g. *E. coli*), or in a
eukaryotic host (e.g., *S. cerevisiae* or mammalian cells,
e.g. COS1, CHO, NIH3T3, and JEG3 cells, or in the cells
of an arthropod, e.g. *S. frugiperda*). Such cells are
available from e.g. the American Type Culture Collection,
Manassas, VA. The method of transfection and the choice
of expression vehicle will depend on the host system
selected. Transformation and transfection methods are
described, e.g. in Sambrook et al. *supra*; expression
vehicles can be chosen from those provided e.g. in
Cloning Vectors: A Laboratory Manual P.H. Powels et al
(1985), Supp. 1987.

For most of the amino acids used to build
proteins, more than one coding nucleotide triplet (codon)
can code for a particular amino acid residue. This
property of the genetic code is known as redundancy.
Therefore, a number of different nucleotide sequences can
code for a particular subject biosurfactant peptide e.g.,
Lactobacillus biosurfacnt peptide. The present invention
also contemplates a deoxyribonucleic acid (DNA) molecule
or segment that defines a gene coding for, i.e., capable
of expressing, a subject peptide or a subject chimeric
peptide from which a peptide of the present invention may
be enzymatically or chemically cleaved.

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DNA molecules that encode peptides of the
present invention can be synthesized by chemical
techniques, for example, the phosphotriester method of
Matteuccie, et al., J. Am. Chem. Soc. 103:3185(1981).
Using a chemical DNA synthesis technique, desired
modifications in the peptide sequence can be made by
making substitutions for bases which code for the native
amino acid sequence. Ribonucleic acid equivalents of the
above described DNA molecules may also be used.

A nucleic acid molecule comprising a vector
capable of replication and expression of a DNA molecule
defining coding sequence for a subject polypeptide or
subject chimeric polypeptide is also contemplated.

The peptides of the present invention are
chemically synthesized by conventional techniques such as
the Merrifield solid phase technique. In general, the
method comprises the sequential addition of one or more
amino acid residues to a growing peptide chain.
Normally, either the amino or carboxyl group of the first
amino acid residue is protected by a suitable,
selectively removable protecting group. A different,
selectively removable protecting group is utilized for
amino acids containing a reactive side group such as
lysine.

A preferred method of solid phase synthesis
entails attaching the protected or derivatized amino acid
to an inert solid support through its unprotected
carboxyl or amino group. The protecting group of the
amino or carboxyl group is then selectively removed and
the next amino acid in the sequence having the
complementary (amino or carboxyl) group suitably
protected is admixed and reacted under conditions

suitable for forming the amide linkage with the residue
already attached to the solid support. The protecting
group of the amino carboxyl group is then removed from
this newly added amino acid residue, and the next amino
acid (suitably protected) is then added, and so forth.
After all the desired amino acids have been linked in the
proper sequence, any remaining terminal and side group
protecting groups including the solid support are removed
sequentially or concurrently to yield the final peptide.
The lyophilized oligopeptides are resuspended in double
distilled H_2O at 2 mg/ml as stock solutions and
subsequently diluted in M199-HPS for experiments.

Peptides SEQ ID NOS:1-6 have the following sequences:

SEQ ID NO:1 Xaa_1-Q-G-Xaa_2-V-S-Xaa_3-Xaa_4-G-S-C-T-C-A-Q-K
SEQ ID NO:2   E-V-Y-Xaa_1-F-Q-E-Xaa_2-Xaa_3-R
SEQ ID NO:3   W-N-Y-V-S
SEQ ID NO:4   R-A-F-A-Xaa_1-Xaa_2-S-N-W-P-V-K
SEQ ID NO:5   G-Xaa_1-Q-Xaa_2-Xaa_3-D-Xaa_4-Xaa_5-Xaa_6-Xaa_7-
                Xaa_8-R
SEQ ID NO:6   G-Xaa_1-Q-N-D-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Xaa_6-R
including analogs and fragments which maintain anti-
pathogen activity; wherein
          A = Ala = Alanine
          R = Arg = Arginine
          N = Asn = Asparagine
          D = Asp = Aspartic acid
          B = Asx = Asparagine or aspartic acid
          C = Cys = Cysteine
          Q = Gln = Glutamine
          E = Glu = Glutamic acid

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Z = Glx = Glutamine or Glutamic acid

G = Gly = Glycine

H = His = Histidine

I = Ile = Isoleucine

5   L = Leu = Leucine

K = Lys = Lysine

F = Phe = Phenylalanine

P = Pro = Proline

S = Ser = Serine

10   T = Thr = Threonine

W = Trp = Tryptophan

Y = Tyr = Tyrosine

V = Val = Valine

X = Xaa_1 = Any amino acid

15   Consistent with the observed properties of the
peptides of the invention, the present peptides can be
used to inhibit, prevent, treat or reduce the risk of
pathogen-induced infection in animals, including humans.
Organisms which can infect the host, including *S. aureus*

20   (particularly methicillin-resistant - MRSA), *S.
epidermidis* (particularly methicillin-resistant - MRSE) ,
Pseudomonas aeruginosa, Enterococcus faecalis (including
vancomycin-resistant - VRE), *Bacteroides sp.* and certain
viruses bind to collagen receptors and thus can be

25   particularly detrimental in wounds and surgical implants.
Accordingly, the present invention contemplates methods
to block, interrupt or otherwise prevent the association
of pathogenic organisms to collagen receptors and thereby
effectively inhibit, prevent and/or treat infections.

30   In another embodiment, the peptides of the
present invention can be provided in consumer products
such as cellophane wraps, sandwich bags, bottle tops,

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food preparation trays to block or inhibit pathogens from binding to the polymers or materials used to manufacture such consumer products.

In still another embodiment, beneficial flora from a subject suffering from an infection is identified and by products produced therefrom are detected and proteins or peptides are then isolated from the by-products, e.g., biosurfactants. The proteins or peptides isolated from the by-products are e.g., exogenously applied to the subject to reduce the risk of infection and to eradicate or inhibit such infection.

In still yet another embodiment of this invention, a diagnostic system is provided which identifies organisms within a subject, which organisms express proteins, peptides, glycoproteins or amino acids which function as blockers or inhibitors of pathogen binding. The diagnostic system used to identify the desired indigenous microorganisms includes singly or in combination SELDI (protein ID), molecular typing, PCR and other conventional tools. For example, a strain of *Staphylococcus epidermidis* present on the skin of a subject or *Lactobacillus* in the intestine of a subject which expresses a desired protein is detected in accordance with the present invention. Prebiotics (nutrients which stimulate the normal flora but do not stimulate pathogens), functional foods or other substances, are introduced to enhance the presence of the strain which produces the blocking substances, e.g., peptides and in this way reduces the risk of pathogenic infection in the host.

If the patient is susceptible to infection by various pathogens, such as multi-drug resistant

staphylococci, vancomycin resistant enterococci and
Klebsiella, a combination of blocking proteins, peptides,
glycoproteins or amino acids is introduced to confer
protection. The present invention also contemplates
stimulation of the normal flora in the intestine (a
common source of infecting organisms), vagina, oral
cavity, respiratory tract, skin or other sites.

In another embodiment of the present invention,
one or more biosurfactant peptides are included in
pharmaceutical compositions.

Preferably, compositions containing the
biosurfactant peptides of the present invention are
administered topically (i.e. directly to the site of a
wound or the skin, tissue or medical biologically
compatible devices), as a spray, as an integral part of a
bandage, as an adjunct to antibiotic or antiviral or
antifungal therapy or in liposomes or other delivery
vehicles, or intravaginally, intrarectally, orally or
intravenously to inhibit, prevent or treat infection.
When administered intravenously, the peptide compositions
may be combined with other ingredients, such as carriers
and/or adjuvants. The peptides may also be covalently
attached to a protein carrier, such as albumin, so as to
minimize clearing of the peptides. There are no
limitations on the nature of the other ingredients,
except that such ingredients must be pharmaceutically
acceptable, efficacious for their intended administration
and cannot degrade the activity of the active ingredients
of the compositions. Pharmaceutical compositions
containing the biosurfactant peptides suitable for oral
administration may be incorporated in foods or nutrients

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ingested to interfere with pathogen colonization and
spread in the gut.

     The pharmaceutical forms suitable for injection
include sterile aqueous solutions or dispersions and
sterile powders for the extemporaneous preparation of
sterile injectable solutions or dispersions. In all
cases the ultimate solution form must be sterile and
fluid. Typical carriers include a solvent or dispersion
medium containing, for example, water buffered aqueous
solutions (i.e., biocompatible buffers), ethanol, polyols
such as glycerol, propylene glycol, polyethylene glycol,
suitable mixtures thereof, or vegetable oils.
Sterilization can be accomplished by any art-recognized
technique, including but not limited to, filtration or
addition of antibacterial or antifungal agents, for
example, paraben, chlorobutanol, phenol, sorbic acid or
thimerosal. Further, isotonic agents such as sugars or
sodium chloride may be incorporated in the subject
compositions.

     Production of sterile injectable solutions
containing the subject peptides is accomplished by
incorporating these compounds in the required amount in
the appropriate solvent with various ingredients
enumerated above, as required, followed by sterilization,
preferably filter sterilization. To obtain a sterile
powder, the above solutions are vacuum-dried or freeze-
dried as necessary.

     When the peptides of the invention are
administered orally, the pharmaceutical compositions
thereof containing an effective dose of the peptide can
also contain an inert diluent, as assimilable edible
carrier and the like, be in hard or soft shell gelatin

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capsules, be compressed into tablets, or may be in an
elixir, suspension, syrup or the like.

The subject peptides are thus compounded for
convenient and effective administration in
pharmaceutically effective amounts with a suitable
pharmaceutically acceptable carrier in a therapeutically
effective dose.

The peptides should preferably be administered
in an amount of at least about 100 ng per dose, more
preferably in an amount up to about 5 mg to about 2 grams
per dose. Since the peptide compositions of this
invention will eventually be cleared from the
bloodstream, re-administration of the compositions is
indicated and preferred.

The peptides can be administered in a manner
compatible with the dosage formulation and in such amount
as well be therapeutically effective. Systemic dosages
depend on the age, weight and conditions of the patient
and on the administration route. For example, a suitable
dose for the administration to adult humans ranges from
about 5 mg to about 2 g per kilogram of body weight.

As used herein, a pharmaceutically acceptable
carrier includes any and all solvents, dispersion media,
coatings, antibacterial and antifungal agents, isotonic
agents the like. The use of such media and agents are
well-known in the art. The pharmaceutically acceptable
carriers used in conjunction with the peptides of the
present invention vary according to the mode of
administration. For example, the compositions may be
formulated in any suitable carrier for oral liquid
formulation such as suspensions, elixirs and solutions.

Compositions for liquid oral dosage include any of the usual pharmaceutical media such as, for example, water, skim milk, yogurt, oils, alcohols, flavoring agents, preservatives, coloring agents and the like. In the case of oral solid preparations (capsules and tablets) carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like may be used. In addition, carriers such as liposomes and microemulsions may be used.

In a further aspect of the present invention, the pharmaceutical compositions of the present invention are employed for the treatment of infection. Thus, the present invention provides methods of treating an infection in a subject by administering a therapeutically effective amount of a pharmaceutical composition of the present invention.

The term "therapeutically effective amount" means the dose required to treat an infection.

By "infection" is meant a pathological disorder, the onset, progression or the persistence of the symptoms of which requires the participation of one or more biosurfactant peptides.

The term "treatment" or "treat" refers to effective inhibition, prevention or treatment of the infection.

The term "subject" refers to any mammalian subject. Preferably, the subject is a human.

The invention is further illustrated by the following specific examples which are not intended in any way to limit the scope of the invention.

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EXAMPLE 1

SELDI (surface enhanced laser desorption/ionization) was used to separate, detect and analyze native proteins without using labeling or time consuming biochemical analytical systems. The SELDI system was used to quickly and accurately determine whether clinically important strains of lactobacilli expressed collagen binding proteins.

Four *Lactobacillus* strains were tested. *L. fermentum* RC-14 was selected because of its potent biosurfactant inhibitory activity against many pathogens. *L. rhamnosus* GR-1 and 36 also produce biosurfactants, and were also inhibitory to enterococci.

The organisms were grown in MRS broth overnight, harvested and the biosurfactant isolated by incubating the organisms for two hours at room temperature.

EXAMPLE 2

Animal Housing

        Eight week-old (300 gram) male Sprague-Dawley
rats (Charles River Inc, Montreal QC) were housed in
5   shoebox plastic cages (2 animals per cage), maintained on
a 12-hour light cycle, and given free access to standard
rodent chow and water. Following surgery, the animals
were examined daily for clinical signs of infection.

10   **Bacterial Cultures**

        Staphylococcus aureus (Oxford strain) was
cultured in Brain Heart (BH) growth media overnight at
37°C and plated on BH agar plates to determine the
colony-forming unit (CFU) activity of the bacteria.
15   Following CFU measurements *S. aureus* (SA) suspensions
were diluted in phosphate buffered saline (PBS) to attain
the correct number of CFU for subsequent surgical implant
inoculation *in vivo*. In a similar procedure,
Lactobacillus fermentum RC-14 and *Lactobacillus*
20   *rhamnosus* GR-1 were cultured using MRS Broth and MRS agar
plates. CFU measurements were taken and appropriate PBS
dilutions prepared for surgical implant inoculations *in*
vivo.

25   **Biosurfactant Production and Isolation from *Lactobacillus***
fermentum RC-14 cultures

        Crude biosurfactant (BSF) was collected from
lactobacilli strains GR-1 and RC-14 as previously
described (Reid, G., C. Heinemann, M. Velraeds, H. C. van
30   der Mei, and H. J. Busscher. 1999. Biosurfactants
produced by *Lactobacillus*. Methods in Enzymol. 310: 426-

432, incorporated herein by reference). Bacterial cells
were harvested by centrifugation (10,000 x g, 10 min,
10°C), washed twice in demineralized water and re-
suspended in PBS. The *Lactobacilli* were then placed at
room temperature and gently stirred for 2 hr to trigger
the release of the biosurfactant.. The solution was then
centrifuged (10,000 x g, 10 min, 10°C), and the
supernatant collected, filtered (0.22mm) and dialyzed
against double demineralized water at 4°C, using
spectrapor dialysis tubing (6-8 kDa MW cutoff). The
dialyzed substance was either used directly or freeze-
dried (-10°C / -5uM Hg / 1-2 days) and stored at -20°C.
Protein concentrations were determined using a BCA
protein assay kit (Pierce). For surgical implant
experiments the biosurfactant was suspended in sterile
PBS (2mg/ml) and incubated with the surgical implants for
12 hours at 4°C prior to placing them in the animals.

Animal Surgery:

Sprague-Dawley rats were anaesthetized via
peritoneal injection of a mixture of hydrochloride-
ketamine (100 mg/ml) and xylazine (10mg/ml) at the rate
of 0.1ml per 100g of body weight. Each anaesthetized rat
was clipped of dorsal hair at the surgical site and
liberally swabbed with a povi-iodine antiseptic solution.
A single 2cm incision was made along the dorsal skin. A
single (1cm x 1cm x 0.5 mm) sterile silicone implant
(Dagnone Inc.) was then inserted into the subcutaneous
pocket adjacent to the skin incision and co-inoculated
with the indicated number of *S. aurues* CFU (Figure 1) and
the indicated amount of either RC-14 biosurfactant
peptides, bovine serum albumin (BSA), or recombinant His-

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tagged p29CnB. Surgical incisions were then closed with
3.0 polygalactin-coated 910 (vicryl) interrupted sutures
and animals treated with post-operative analgesic
(buprenorphine hydrochloride, 0.01 mg/kg subcutaneously).

5

S. aureus CFU measurements from Surgical implants

At the specified post-operative times, animals
were sacrificed by CO_2 asphyxiation. Surgical sites were
opened and immediately assessed for signs of acute
10   inflammation and infection. Silicone implants and the
surrounding tissues were harvested. Wound fluid was also
collected from infected surgical sites and stored at
-80°C. Surgical implant associated bacteria were harvested
by sonication and bacterial numbers quantified using
15   standard microbiological techniques. Implants were rinsed
in PBS and then subjected to a 30-second sonication
treatment. PBS diluted bacterial suspensions were then
plated on either MRS (lactobacilli), or BH (*S. aureus*)
agar plates and incubated overnight at 37°C under
20   anaerobic (5% CO_2) or aerobic conditions, respectively.

Gram Staining of Implants

Harvested implants were analyzed using a Gram
staining kit (CMS protocol, Fisher) in order to assess
25   both the extent of bacterial colonization of the implants
and the efficiency of harvesting the implant adherent
bacteria by sonication. The staining procedure included:
Crystal violet (1 min), iodine solution (1 min),
decolorizing agent (30 sec), safranin (1 min). Implants
30   were then imaged using a light microscope (Axiophot,
Zeiss).

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Polymerase Chain reaction (PCR)

The PCR technique was used to verify the
species of bacteria obtained from each surgical implant.
Briefly, bacteria colonies were picked, briefly washed in
a Tris-EDTA buffer (pH 7.4), lysed in 10% SDS for 30 min.
centrifuged (4000 rpm, 5min) and the resulting pellet
heated in a 300 Watt-microwave (Hi power) for 5 minutes.
DNA was isolated from each pellet via phenol / chloroform
/ isoamyl alcohol (24:23:1) (pH 8.0) extraction (1x),
followed by ethanol precipitated. The DNA was then added
to a PCR reaction mixture containing: 0.5mM specific
oligo-nucleotide primers (*Staphylococcus aureus* forward
primer = 5'- GAAGTCGTAACAAGG - 3' (SEQ ID NO:6) and
reverse primer = 5'- CAAGGCATCCACC GT - 3'(SEQ ID NO:7);
L. fermentum RC-14 forward primer = 5'-
AAACTTTCTTATTCTATTCT GGT -3' (SEQ ID NO:8) and reverse
primer = 5' - AACTGATTCGTCCCGTAAA-3' (SEQ ID NO:9); *L.
rhamnosus* GR-1 forward primer = 5'-ACGAGGC AC-3', reverse
primer = 5'-ACGCGCCCT-3'), 0.3mM dNTP, 2mM $MgCl_2$, and 1
unit of Platinum Taq Polymerase (Gibco-BRL) in a final
reaction volume of 50ml. PCR thermal cycling (Model 212
Lab-line) parameters included: 1 denaturation cycle (2
min / 94°C), followed by either 25 amplification cycles
(denaturation 1 min / 94°C, annealing 7 min / 52°C, and
extension 2 min / 68°C) for *S. aureus*, or 40
amplification cycles (denaturation 1 min / 94°C,
annealing 2 min / 52°C or 35°C and extension 2 min / 68°C)
for RC-14 and GR-1 respectively. This was followed by a
final extension cycle of 10 min at 68°C. The PCR products
were separated by agarose (2%) gel electrophoresis,
visualized by ethidium bromide staining and photographed
under UV transillumination.

Isolation of collagen-binding proteins from *L. fermentum*
RC-14 using collagen affinity columns

Lactobacillus ferementum RC-14 biosurfactant
(4mg) was applied to either a collagen type-I (rat tail,
5   Sigma) or collagen type-III (bovine skin, Sigma)
polystyrene alkylamine bead affinity column as per the
manufacturers advice (Pierce). Briefly, 100 hydrazide
derivatized ¼ inch diameter spherical polystyrene beads
(Pierce), were soaked in 50mM phosphate buffer (pH 6.0)
10   containing 10gm of succinic anhydride for 15hr at room
temperature. The beads were then washed extensively in
distilled water and then treated with a XmM EDC (1-
Ethyl,3,3-dimethylaminopropyl-carbodiimide HCl) solution
(pH 10) 2 hours at room temperature. The beads were then
15   washed with distilled water and then incubated overnight
at 4°C with a PBS solution containing 5mg (total) of
either Collagen I or Collagen III. The beads were then
packed into a 30cc syringe column and then equilibrated
with a 3 bed volumes of PBS (pH 7.4). Biosurfactant (4mg)
20   was applied to the column and the resulting flow-through
re-applied (5x) to allow for optimal protein binding.
Flow-though fractions were then collected and the column
washed with 2 bed volumes of PBS. Bound proteins were
then eluted with a PBS solution containing 1M NaCl
25   fractions. All fractions were analyzed for protein
concentration using a BCA kit (Pierce), and dialyzed
overnight at 4°C (Spectra, 5000 MW cutoff) against PBS.
Dialyzed column fractions were then used to coat sterile
silicone implants and to inoculate the surgical wound
30   site as described above.

Over expression and purification of recombinant p26

The gene encoding for p29CnB was subcloned into the BamHI-XhoI multicloning site of the bacterial expression vector pET-30 (Novagen, USA). BLR (DE3) competent cells (Novagen, USA) were transformed with the engineered p29CnB-His/pET-30 expression vector. Culture (LB broth + 30ug/ml kanamycin) of a p29CnB-His/pET-30 expressing clone was induced to over express the dual His-tagged recombinant protein by the addition of 1mM IPTG (overnight at 37°C). The "His-Bind" purification kit from Novagen was used and the manufacturers instructions were followed in order to purify His-tagged recombinant protein. Proteins were removed by centrifugation (30min, 6000 rpm, Sorvall RC-3B) and collected in the resulting culture media, which contained the sought after secreted recombinant protein. The collected media was then applied to a small polypropylene column containing 2.5ml of packed, charged (50 mM NiSO$_4$) and equilibrated (binding buffer: 5 mM imidazole, 0.5 M NaCl, 20 mM Tris·HCl pH 7.9) His-Bind resin (Novagen, USA). After an initial wash step with binding buffer the His-tagged protein was eluted using a 20mM Tris·HCl (pH 7.9) buffer containing 0.5 M NaCl and 60mM imidazole. The eluted fractions were then pooled, dialysed against dH$_2$O, lyopholized and stored at -20°C. Protein purity was analyzed by SDS-PAGE.

Preactivated Surface (PS-1) ProteinChip® analysis and SELDI-Qq-TOF amino acid sequencing of collagen binding proteins isolated from RC-14

Aliquots (2-3µl) of RC-14 or GR-1 biosurfactants were spotted onto PS-1 ProteinChip®

(Ciphergen, Calif) arrays that contained either
immobilized collagen type-I or collagen type-III. The
SELDI spectra of the collagen binding proteins was then
examined. The biosurfactant proteins bound to each of
5   the spots on the collagen cross-linked PS-1 arrays were
then directly subjected to trypsin digestion. The on-chip
tryptic digests were then analyzed using a prototype
SELDI-interfaced SIEX Q-star. Figures 3a and 3b show the
tryptic profiles of the collagen binding fractions of RC-
10   14 and GR-1 biosurfactants respectively, with the labeled
peaks being chosen for subsequent MS/MS sequence
analysis. Shown in Figures 3c, 3d, 3e are the MS/MS
spectra of three unique tryptic peptides derived from the
RC-14 biosurfactant peptide map (Figure 2a). In total 5
15   unique peptide sequences were identified using either Cn-
I/PS-1 or Cn-III/PS-1 arrays (see Table 1).

20

EXAMPLE 3

Lactobacillus fermentum RC-14 collagen binding activity
inhibited *in vivo* implant infection by *S. aureus.*

The ability of lactobacilli biosurfactants to
prevent uropathogens from adhering and subsequently
infecting healthy human tissue is believed to account, in
part, for the beneficial clinical effects of these
probiotics. Based on this notion we determined that
Lactobacillus fermentum RC-14 biosurfactant produces a
number of collagen binding proteins using SELDI-TOF-MS
(Howard, J., et al.. Identification of collagen binding
proteins in *Lactobacillus* using advanced ProteinChip
technology. Appl. Environ. Microbiol. In press.).
Collagen is a major extracellular matrix (ECM) component
of tissues that has also been shown to be an important
site of attachment for invading pathogenic bacteria, like
Staphylococcus aureus.

To determine whether or not the collective
collagen-binding activity of *L. fermentum* RC-14 may be
responsible for its recently discovered anti-infective
effects *in vivo* (United States Patent Application Serial
No. 09/579,306, filed May 25, 2000, incorporated herein
by reference), the RC-14 collagen-binding peptides were
isolated using a traditional affinity column purification
approach. Collagen types I and III were chemically cross-
linked to alkylamine beads (Pierce) according to the
manufacturers specification. These collagen types were
chosen since they are the most common collagens found
within the skin. To ensure the effectiveness of the
collagen affinity columns, the binding affinity of a
recombinant collagen binding protein, p29CnB, which was
recently purified from RC-14 biosurfactant was tested

first (Heinemann, C., et al. Purification and characterization of a biosurfactant produced by *Lactobacillus fermentum* RC-14 inhibiting *Enterococcus faecalis* 1131 adhesion. FEMS Microbiol. Letts. In press.). As shown in Figure 1a, bacteria were engineered to express a recombinant His-tagged form of p29CnB, which was then purified from the culture media using a Ni^{+2} affinity column (His-Resin kit, Novagen, USA). As shown in Figure 1a, all 4mg of the recombinant p29CnB used in the experiment bound very tightly to both Cn-I and Cn-III affinity columns, thereby confirming the effectiveness of this purification scheme. Next, the collagen binding peptides from the crude RC-14 biosurfactant were purified and their activity assayed *in vivo* using the rat surgical implant model. As shown in Figure 1b, 100µg of protein eluted from the Cn-I column (Cn-I EF) inhibited *S. aureus* induced surgical implant infection by 80%, which was the same amount of anti-infection activity observed for crude RC-14 biosurfactant. By comparison, 100µg of the Cn-III elution fraction (Cn-III EF) inhibited *S. aureus* infection by 65%, while the flow through fractions from both types of collagen affinity columns inhibited infection by 20%. In addition, all four column fractions could significantly inhibit the number of *S. aureus* CFUs that adhered to the surgical implants, when compared to the BSA control treated group (Figure 1c). However, the most significant anti-adhesive effect was produced by the Cn-I binding fraction of RC-14 biosurfactant, followed by crude RC-14 biosurfactant and the Cn-III binding fraction (P = 0.00027, P = 0.00006, P = 0.0002, respectively).

EXAMPLE 4

Identification of RC-14 BSF collagen binding peptides using PS-1 ProteinChip® arrays and SELDI-Qq-TOF.

5   The RC-14 biosurfactant was screened for peptides possessing collagen-binding activity, using *Surface Enhanced Laser Desorption/Ionization* time of flight mass spectrometry (SELDI-TOF-MS). Recently, with the aid of this novel ProteinChip® technology (Ciphergen

10   Biosystems, Inc.), it has been demonstrated that RC-14 biosurfactant contains a number of collagen binding proteins and peptides (Howard *et al.* in press). In an effort to definitively identify these individual collagen-binding proteins and peptides, 'on-chip'

15   protease digest of the partially purified RC-14 biosurfactant was performed, followed by MS-MS sequencing of the peptide fragments using SELDI-Qq-TOF. Binding of either the RC-14 or control GR-1 biosurfactant to PS-1 ProteinChip® arrays that contain either immobilized

20   collagen type-I or type-III occured first. Shown in Figure 2a is a typical SELDI protein spectrum of the RC-14 biosurfactant that bound to a Cn-III/PS-1 array. The spectra of the collagen binding proteins for both Cn-I/PS-1 and Cn-III/PS-1 arrays were virtually identical

25   (data not shown). The RC-14 proteins bound to each of the spots on the collagen cross-linked PS-1 arrays were then directly subjected to trypsin digestion. The 'on-chip' tryptic digests were then analyzed using a prototype SELDI-interfaced SIEX Q-star. Figures 3a, 3b show the

30   tryptic profiles of the collagen binding fractions of RC-14 and GR-1 biosurfactants, respectively, with the

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labeled peaks being chosen for subsequent MS/MS sequence analysis. Shown in Figures 3c,d,e, are the MS/MS spectra of three unique tryptic peptides derived from the RC-14 peptide map (Figure 2a). In total 5 novel peptide sequences were identified using either Cn-I/PS-1 or Cn-III/PS-1 arrays (Table 1).

TABLE 1

SELDI-Qq-TOF MS/MS amino acid sequencing of tryptic peptides from Cn-I/PS-1 and Cn-III/PS-1 arrays

PS-1 array	Tryptic peptide (Da.)	Sequence
Cn-I/PS-1	1. 1465.731 2. 1308.635 3. 1191.569	L^QGLVS[FA]*GSCTCAQK EVYLFQE[QP]R WNYVS
Cn-III/PS-1	1. 1329.685 2. 1263.696	RAFALLSNWPVK GLQGG¶DL[SH][SH]R

* [] bracketed amino acids indicate the tentative order of the sequence.

^ Underlined <u>amino acids</u> indicate likely identities (e.g., M.W. of leucine and isoleucine are the same).

¶ Potentially <u>GG</u> = N (Asparagine) since they have the same molecular weight (M.W.).

WHAT IS CLAIMED IS:

    1.   An isolated biosurfactant peptide comprising Xaa_1-Gln-Gly-Xaa_2-Val-Ser-Xaa_3-Xaa_4-Gly-Ser-Cys-Thr-Cys-Ala-Gln-Lys (SEQ ID NO:1), wherein Xaa_1 and Xaa_2 is any amino. acid and Xaa_3 and Xaa_4 are selected from the group consisting of Phe or Ala.

    2.   An isolated biosurfactant peptide comprising Leu-Gln-Gly-Leu-Val-Ser-Xaa_3-Xaa_4-Gly-Ser-Cys-Thr-Cys-Ala-Gln-Lys (SEQ ID NO:1), and Xaa_3 and Xaa_4 are selected from the group consisting of Phe or Ala.

    3,   An isolated biosurfactant peptide comprising Glu-Val-Tyr-Xaa_1-Phe-Gln-Glu-Xaa_2-Xaa_3-Arg (SEQ ID NO:2), wherein Xaa_1 is any amino acid and Xaa_2 and Xaa_3 are selected from the group consisting of Gln or Pro.

    4.   An isolated biosurfactant peptide comprising Trp-Asn-Tyr-Val-Ser (SEQ ID NO:3).

    5.   An isolated biosurfactant peptide comprising Arg-Ala-Phe-Ala-Xaa_1-Xaa_2-Ser-Asn-Trp-Pro-Val-Lys (SEQ ID NO:4), wherein Xaa_1 and Xaa_2 can be any amino acid.

    6.   An isolated biosurfactant peptide comprising Gly-Xaa_1-Gln-Xaa_2-Xaa_3-Asp-Xaa_4-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Arg (SEQ ID NO: 5), wherein Xaa_1, Xaa_2, Xaa_3 and Xaa_4 can be any amino acid and Xaa_5, Xaa_6, Xaa_7, and Xaa_8 are selected from the group consisting of Ser or His.

    7.   An isolated biosurfactant peptide comprising Gly-Xaa_1-Gln-Asn-Asp-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Xaa_6-Arg (SEQ ID NO:6), wherein Xaa_1 and Xaa_2 is any amino acid and Xaa_3 Xaa_4, Xaa_5 and Xaa_6 are selected from the group consisting of Ser or His.

    8.   An isolated nucleic acid molecule coding for any one of the peptides of Claims 1-7.

9. A pharmaceutical composition comprising the peptide of any of Claims 1-7, and a pharmaceutically acceptable carrier.

10. A method of treating an infection in a subject, comprising administering to the subject a therapeutically effective amount of the peptide of any of Claims 1-7 and a pharmaceutically acceptable carrier.

11. A method of inhibiting the binding of a pathogen to a collagen receptor comprising the administration of at least one peptide of any one of Claims 1-7.

12. A biosurfactant peptide.

13. A pharmaceutical composition comprising a biosurfactant peptide and a pharmaceutically acceptable carrier.

14. A method of inhibiting an infection comprising blocking the interaction of a pathogen with a collagen receptor by the administration of a biosurfactant peptide.
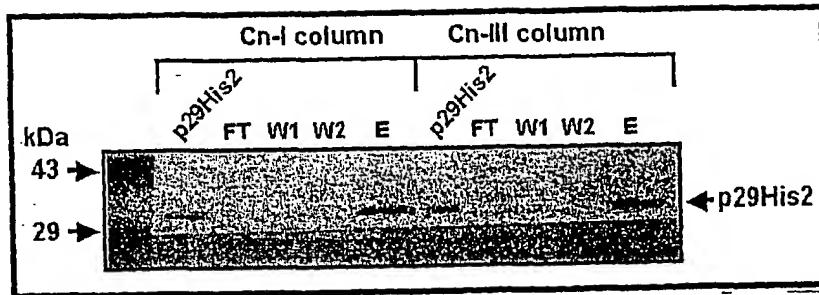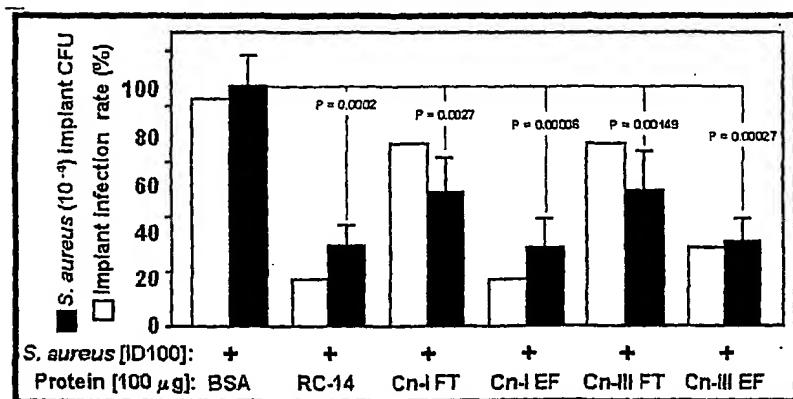
FIGURE 1A

FIGURE 1B
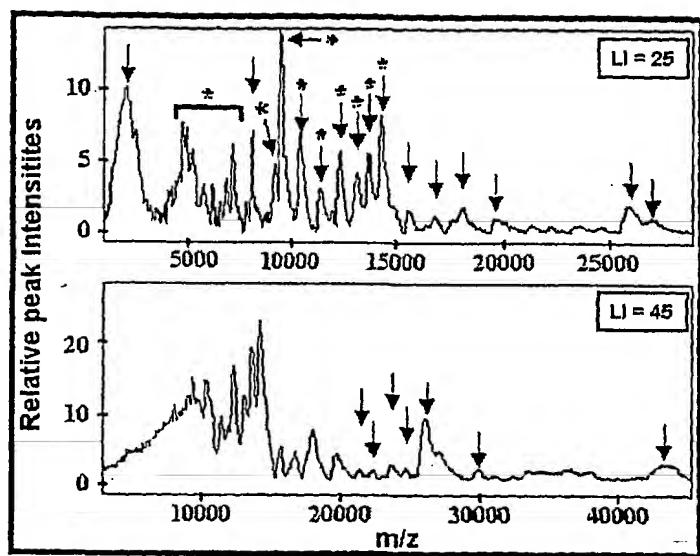
FIGURE 2
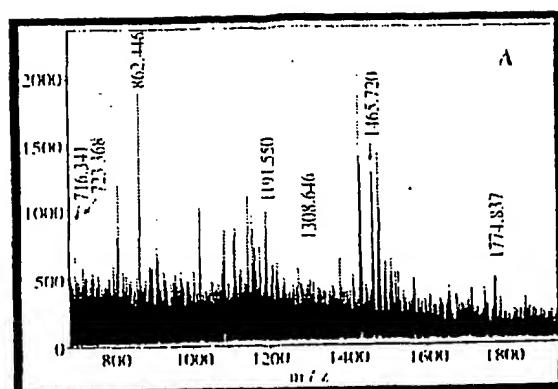
FIGURE 3A
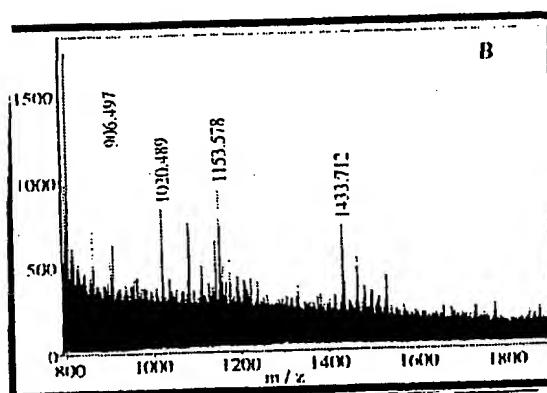
FIGURE 3B
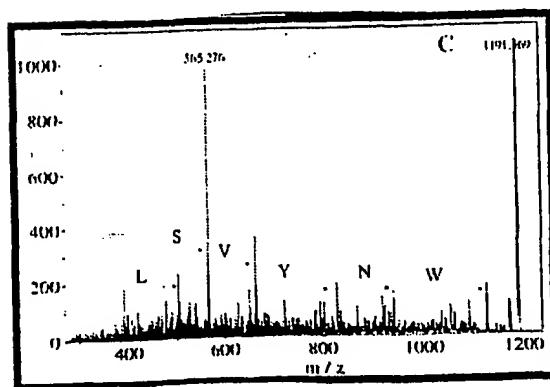
FIGURE 3C

FIGURE 3D

FIGURE 3E